5/5/80 DER #5

Fluvalinate: Developmental Toxicity Study in Rats (with Pilot Study) Zoecon Corporation. 1980. MRID No. 00077026, 00077027, 92069054. HED Doc. No. 001786.

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Subject: Teratology Study in Rats: Final Report

Test Compound: ZR-3210 Technical (Racemic Mixture)
MAVRIK(R) Technical
fluvalinate

Accession No.: 070097

Testing Facility: Hazleton Laboratories America, Inc., Vienna, Virginia

Project Number: 777-130

Responsible Professionals:

Ruth S. Durloo - Research Assistant
Marjorie Kane - Group Leader
Kenneth Bristol - Technical Writer
Alan Hoberman - Study Director
Frederick Reno - Director, Scientific Resources Department

Testing Period: January 3, 1980 - January 29, 1980

Report Submitted to Sponsor: May 5, 1980

Purity of Test Material: 93.8%

Batch or Lot Number: Run 7-Anal. No. 0979-069

Stability: Stable for at least six months when stored in sealed glass containers and exposed to artificial light at 25 and 42°C.

Stable in corn oil when heated to 60°C.

Stable in corn oil for at least 5 months when stored at laboratory ambient conditions (20°C and fluorescent light) in glass containers at concentrations of 5 and 10 mg/ml.

Materials and Methods: Appropriate amounts of technical ZR-3210 (adjusted to 100% of active ingredient for purposes of dosage calculation) were weighed and mixed with Mazola Corn Oil(R) and stirred on a magnetic stirrer at 60°C until a solution was formed. The test solutions were prepared at concentrations of 5.0 and 10.0 mg/ml. The 5.0 mg/ml solution was used to dose the low-dose group, and the 10.0 mg/ml solution was used to dose the mid- and high-dose group. Samples were also forwarded to the sponsor for analysis.

One hundred ten female and 55 male sexually mature rats of the Sprague-Dawley strain were shipped from A.R.S. Sprague-Dawley, Madison, Wisconsin. The animals were acclimated to laboratory conditions for sixteen days prior the beginning of the mating phase. A staff veterinarian examined the animals during this period.

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Animals were mated by placing one male with two females per breeding cage. A vaginal examination of each female was performed daily during the mating period. The day that sperm (microscopic examination of a saline extract drawn from the vagina) or a vaginal plug were observed (preventing vaginal examination for sperm) was designated Day 0 of gestation. Female body weights were taken on Day 0 of gestation and ranged from 210 to 277 grams. The day that sperm or a vaginal plug was observed, females were randomized into one of four groups until a total of 25 females per group were formed. Extra animals, including the males after breeding, were subsequently discarded. Water and food (Purina Rodent Laboratory Chow(R)) were avaiable ad libitum throughout the study. Food and water were also analyzed for select contaminants, but were not included in the report.

All females placed on test were uniquely identified by ear tags and were housed individually in polycarbonate boxes marked with corresponding animal numbers. Groups 1, 2, 3, and 4 were designated, respectively, as the vehicle control, low-, mid-, and high-dose groups and received either corn oil or 2, 10, or 50 mg/kg of test compound in the corn oil vehicle. The solutions were administered at approximately the same time each day by oral gavage from Day 6 through Day 15 of gestation. All doses were based on Day 6 body weight.

All of the females were sacrificed by carbon dioxide asphyxiation on Day 19 of gestation. The reproductive tract weight of each pregnant rat was excised and weighed. The fetuses were taken by cesarean section and the dams examined for visceral gross pathology.

The following information was recorded for each litter:

- the number of corpora lutea per ovary,
- the number and placement of uterine implantations,
- resorption sites,
- live fetuses, and
- dead fetuses.

Fetuses were removed from the placenta, tagged for identification, examined externally, sexed, and the weight and crown-rump distance (from the front-parietal suture to the base of the tail) were recorded. After the external observations were completed, one-third of the fetuses from each litter were fixed in Bouin's solution, sectioned by Wilson's freehand razor technique (Wilson and Warkany, 1965), and sealed in plastic.

The thorax and abdomen of the remaining two-thirds of the fetuses from each litter were opened with a midline incision and all major organs were inspected in situ. Pups were then eviscerated and placed in 95% ethyl alcohol. After proper fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution, and the fetuses were cleared in a solution of 20% glycerol and then glycerol-ethanol-benzyl alcohol (2:2:1) and stored in glycerol ethanol (50:50) (Hurley, 1965). A small amount of thymol was added to retard bacterial growth. The skeletons were examined for anomalies and degree of ossification with the aid of magnification on a light box.

The maternal body weight gains, reproductive tract weights, and fetal body weights, lengths, and ossification of the control group were compared statistically to the treated groups by Bartlett's test for homogeneity of variance (Bartlett, 1937) and one-way classification analysis of variance (ANOVA) (Snedecor and Cochran, 1967). If significant results were obtained from both Bartlett's test and ANOVA, a multiple pairwise comparison procedure (Games and Howell, 1976) was used to compare the group mean values. If a significant result was not obtained from Bartlett's test, but was obtained from ANOVA, Scheffe's multiple pairwise comparison procedure (Scheffe, 1953) was used to compare the group mean values. With the exception of pregnancy rates which were compared by a chi-square method (Snedecor and Cochran, 1967), the reproduction and viability indices, as well as visceral and skeletal indices, were analyzed by Wilcoxon's non-parametric comparison of group means (Snedecor and Cochran, 1967). All analyses were evaluated at the 5.0% probability level. A value statistically significantly higher than control was designated "S+" and a lower one "S-."

Results: None of the females (pregnant or non-pregnant) died during the course of the study.

The following clinical signs were not observed in control animals at anytime prior to dosing:

stains on fur
bloody crust on the nose
reddened eyelid(s)
discharge from the vagina
hunched appearance
appearance of thinness
salivation
depression

The following signs appeared with approximately equal frequency in all animals for the periods measured:

sores on tail
cloudy eye(s)
alopecia

The following signs generally appeared in treated animals at a greater frequency than in controls during days 6-15 of gestation (dosing period). Generally, these responses were observed most frequently in the highest dose group and approximately equally in the low- and mid-dose groups.

stains on fur
bloody crust or reddened area
around eyes
bloody crust on nose
reddened eyelid(s)
lacrimating eye(s)
discharge from the vagina
hunched appearance
appearance of thinness

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An undetermined number of high-dose animals exhibited salivation and depression on Day 8 for approximately one hour after dosing.

An undetermined number of mid-dose females salivated for approximately one hour after dosing on Day 12.

The following clinical signs persisted through Day 19 of gestation but were reduced by about 50% in the high-dose group and anywhere from 50 to 100% in the low- and mid-dose groups:

stains on fur
bloody crust or reddened area around eye(s)
bloody crust on nose
reddened eyelid(s)
hunched appearance
appearance of thinness
salivation - not in evidence
depression - not in evidence

The following signs may be labeled miscellaneous:

wheezing - one animal
soft feces - two animals
firm nodule in the inguinal area - one animal
discharge from vagina

The pregnancy rate within each of the four groups was as follows:

Group	% Pregnant
1 (vehicle control)	80
2 (2.0 mg/kg)	92
3 (10.0 mg/kg)	84
4 (50.0 mg/kg)	92

Mean maternal body weights and mean maternal body weight changes were recorded and analyzed. No statistically significant mean changes were noted for the low-dose group when compared to controls at any recorded time interval. The mean body weight change for the mid-dose group during the period of dosing (Days 6-15) was statistically signficantly lower than the control group for the same period. Comparisons between controls and mid-dose animals were comparable for all other intervals. The mean body weight change in the high-dose group (50 mg/kg) for Days 6-7, 6-15, and 0-19 was statistically signficantly lower when compared to controls for the same periods.

Maternal gross pathology was not remarkable between groups.

The mean reproductive tract weights (grams) were comparable between controls and the low- and mid-dose groups (60, 56, and 55 grams, respectively). Weights for the high-dose group were, however, statistically significantly lower (46 grams).

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The following tables reflect mean ovarian, uterine, and litter data.

Mean Number of:	Dose Group (mg/kg) 0	2	10	50
Corpora lutea	16.5	15.2	15.7	16.0
Implantations	13.8	12.4	12.7	13.3
Resorptions	0.9	0.4	0.9	1.3
Fetuses - dead	0	0.04	0	0
- alive	13	12	11.8	11.9
Mean Values/Litter Basis				
Implanation efficiency %	85.7	81.3	81.9	83.2
Incidence of resorptons %	6.0	7.2	7.2	10.1
Incidence of fetal death %	0	0.3	0	0
Incidence of fetal viability %	94.1	96.6	92.8	89.9

No statistically significant differences were recorded between treated groups and controls for any parameter reported in the above tables.

Mean male fetal body weight and length for the low- and mid-dose groups were comparable to controls. Mean fetal female body weight and length for the low- and mid-dose groups were also comparable to control groups. However, mean male and female body weight and length in the high dose group were statistically significantly lower when compared to controls.

The sex ratio for the treated groups was comparable to controls.

Visceral Findings: The percentages of those fetuses examined that appeared normal were as follows:

Group (mg/kg)	Percent
1 (vehicle control)	84
2 (2.0)	78
3 (10.0)	82
4 (50.0)	92

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The absolute number and percent incidence of anomolies and variants were not statistically significant between controls and treated groups. Values for the low- and mid-dose groups and controls were within a narrow range. The number of anomolies in the high-dose group was nearly identical to the other groups, which was approximately zero. The number of variants in the high-dose group was substantially lower than in all other groups. Variants in all groups were described as dilated or slightly dilated kidneys or dilated ureters, with the former appearing two to five times more frequently than the latter.

Skeletal Findings: The percentages of those fetuses examined that appeared normal were as follows:

Group (mg/kg)	Percent
1 (vehicle control)	95
2 (2.0)	96
3 (10.0)	98
4 (50.0)	58

The number of anomalies reported for all dose groups and controls was not greater than one anomaly per group.

Skeletal variants were present in all groups but most notably in the high-dose group. The incidence of variants was both statistically significantly greater and approximately 40% greater than in any other group. The examination of animals in the high-dose group revealed large numbers of animals manifesting lagging ossification of the skull, rib cage, vertebral column, pelvic girdle, and extremities. The mean number of sternebrae, caudal vertebrae, and metatarsals and phalanges were all statistically significantly lower than controls. The mean number of metacarpals and phalanges were also much lower than for controls but was not statistically significant.

Discussion: No compound-related signs were observed in the low- and mid-dose groups. Pharmacotoxic signs were seen in the high-dose group. Statistically signficant body weight gain decreases in pregnant dams (an expression of maternal toxicity) was in evidence for the gestational period of the mid-dose (10 mg/kg) group and was greatly accentuated in the high-dose group for the same period. However, the high-dose group (50 mg/kg) initiated the body weight gain decrease as a sharp and severe loss that persisted through day 19 of gestation. The mid-dose maternal group responded to the chemical with an apparent gradual body weight gain decrease and recovered rather quickly upon cessation of dosing. Although food consumption was not reported, the available data suggest an almost immediate anorexia in the high-dose group which this reviewer believes is reflected in the sharp, severe, and persistent body weight gain decrease compared to controls, and accounts for the fetal

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effects described below (e.g., lagging ossification). This rationale is presented as an alternative explanation for the cause of fetal effects as presented by the author, namely that the fetal effects were caused by a delay in or disruption of implantation on days 6-7. However, in the absence of food consumption data, this reviewer does concede that his own opinion is speculative, and he does not totally discount the reason offered by the author as an explanation of the fetal effects.

Pregnancy rates were comparable for all groups, as were the statistical comparisons between all groups for corpora lutea, implantations, resorptions and the number of live/dead fetuses. Mean fetal weight and length for males and females was statistically significantly lower only in the high-dose group and accounts for the statistically significant decrease in the mean reproductive tract weight of this group. Attendant to the small size of the fetuses were numerous observations of a general lack of ossification but no anomolies, either viscerally or skeletally. The general delayed ossification (considered a variant) was observed in the following anotomical structures: skull, rib cage, vertebral column, pelvic girdle, and extremities. This finding was further supported by a statistically significant or substantial decrease in the mean number of sternabrae, caudal vertebrae, metacarpals, metatarsals, and their respective phalanges. It is this reviewer's opinion that the delayed ossification accompanied by small fetal stature without observable visceral or skeletal congenital defects seems to further support a primary cause of anorexia with subsequent secondary effects in the fetus.

Conclusion: Fluvalinate does not appear to be teratogenic under test conditions and the fetotoxic effects observed appear to be secondary to maternal toxicity.

Classification: Core - minimum.

NOEL: Teratology 50 mg/kg (HDT)
Fetotoxicity* 50 mg/kg

*Fetotoxicity secondary to maternal toxicity (delayed ossification, decreased weight and length of fetuses).

LEL: Maternal toxicity 10 mg/kg. Decreased body weight gain.

The following questions have been raised during the course of the review:

1. On page 14 of the report, is the letter "b" in the correct column?

2. Why wasn't food consumption data calculated, reported, and correlated with decreased body weight gain in pregnant females?

3. Are the headings on page 20 described as "number of variants" and "number of anomalies" for the total number of animals showing either anomalies or variants? Please explain.

4. What reason(s) would the sponsor suggest for the substantial decrease in observed visceral variants in the high dose-group?

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Fluvalinate

Page____ is not included in this copy.

Pages 8 through 13 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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racemic

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Subject: Pilot Teratology Study in Rats: Final Report

Test Compound: ZR-3210 Technical (Racemic Mixture)
MAVRIK(R) Technical
fluvalinate

Accession No.: 070097

Testing Facility: Hazleton Laboratories America, Inc., Vienna, Virginia

Project Number: 777-129

Responsible Professionals:

Permelia A. Mossburg - Group Leader
Larry Pence - Reproduction/Teratology Section
Alan Hoberman - Staff Toxicologist
Frederick Reno - Director, Scientific Resources Department

Testing Period: October 24, 1979 - November 16, 1979

Report Submitted to Sponsor: March 12, 1980

Purity of Test Material: 93.8%

Batch or Lot Number: Run 7, Anal. No. 0979-069

Stability: Stable for at least six months when stored in sealed glass containers and exposed to artificial light at 25 and 42°C.

Materials and Methods: Appropriate amounts of ZR-3210 technical (adjusted to 100% active ingredient for purposes of dosage calculation) were weighed, mixed (20 mg/ml) with Mazola Corn Oil(R), and were stirred on a magnetic stirrer at 60°C until a solution was formed. A sufficient volume of each dosing solution for the entire study was prepared initially, and reserved samples were forwarded to the sponsor for analysis. (Note: Analysis indicated that ZR-3210 technical was stable at 60°C in corn oil.)

Fifteen male and 30 female rats of the Sprague-Dawley CD(R) strain were shipped from A.R.S. Sprague-Dawley, Madison, Wisconsin. The body weights for females ranged from 230 to 264 grams. The rats were uniquely identified by ear tags and were individually housed in hanging wire mesh cages. Animal quarters were controlled for temperature, humidity, and a 12-hour light/dark cycle. Purina Rodent Laboratory Chow(R) and water were available ad libitum. All animals were acclimated for a two-week period, during which time they were examined by a staff veterinarian.

Animals were mated by placing one male and two females in a breeding cage. A vaginal examination of each female was performed daily for the presence and viability of sperm. The day of observation of sperm or a vaginal plug was designated as Day Zero of gestation. The mating procedure continued until 20 impregnated females were available. (Note: Although all females mated, not all became pregnant.) The females were then assigned to 4 groups

of 5 females per group. Assignments were made using a table of random numbers. Group 1 served as the control group and received only corn oil. Groups 2, 3, and 4 received, respectively, 10, 50, and 100 mg/kg of the test compound in the corn oil vehicle. The dose volume was kept constant within each group. The test material or corn oil alone was administered daily to females by oral intubation at approximately the same time each day, from Day 6 through Day 15 of gestation. Daily dosage of the test material was based upon the individual rat body weights on the first day of administration.

All of the animals were observed daily for mortality and moribundity. The animals were observed for appearance and behavior on Day 0 and Days 6 through 19 of gestation. Individual body weights were recorded on Days 0, 6, 11, 15, and 19 of gestation.

All surviving rats were sacrificed by carbon dioxide asphyxiation on Day 19 of gestation, and a necropsy was performed. All observed fetuses were removed by cesarean section and the number of corpora lutea, implanation sites, resorptions, and the live and dead fetuses in each uterine horn were recorded. Each fetus was individually removed from the placenta, identified, weighed, externally examined, and sexed; the crown-rump distance was measured and recorded. (Note: Males were anesthetized and sacrificed without necropsy upon completion of mating.)

The ovaries and uterus of each maternal rat were preserved in 10% neutral buffered formalin. Those fetuses with gross observations were tagged and preserved in Bouin's solution.

The maternal body weight gains and mean fetal body weights and lengths of rats in the control group were compared statistically to the treated groups by Bartlett's test for homogeneity of variance (Bartlett, 1937) and the one-way classification analysis of variance (ANOVA) (Snedecor and Cochran, 1967). If significant results were obtained from both Bartlett's test and ANOVA, a multiple pairwise comparison procedure (Games and Howell, 1976) was used to compare the group mean values. If a significant result was not obtained from Bartlett's test, but was obtained from ANOVA, Scheffe's multiple pairwise comparison procedure (Scheffe, 1953) was used to compare the group mean values. The reproduction and viability indices were analyzed by either a chi-square method (Snedecor and Cochran, 1967) or by Wilcoxon's non-parametric comparison of group means. Since only one pregnant rat in Group 4 survived to Day 19 of gestation, Group 4 data were not included in the statistical analysis. All analyses were evaluated at the 5.0% probability level. Statistically signficant values higher than control values were indicated by "S+," those lower by "S-."

Results: Two high-dose animals were found dead during the study, and only one pregnant rat in the high-dose group survived to Day 19 of gestation (see pregnancy rate on next page).

Lacrimating eye(s), nasal discharge, rough haircoat, alopecia around anus, hunched appearance, low food consumption, urine stains, labored respiration, red eyelids, depression, alopecia around eye(s), salivation, and/or stains on fur were noted more frequently in the treated groups than in the control groups.

It was suggested by the authors of the report that some or all of the signs common to control and test animals may have resulted from low humidity in the animal room (T = 71-76°F; RH = 15-74%).

Mean body weights, mean weight changes, and percent changes were calculated for Days 0-6; 6-15, 15-19, and 0-19. Evaluation of the mean body weight rate of change (gain) revealed consistently lower mean changes in the 50 mg/kg dose group for Days 6-19, with a statistically significant lower mean change from Days 0-19. The mean body weight changes of the control and low-dose group (10 mg/kg) were generally comparable. The data in the high-dose group was not analyzed statistically for reasons stated earlier.

Gross necropsy of pregnant dams did not reveal any remarkable pathology attributable to the compound.

The pregnancy rate within each of the four dose groups was as follows:

Group	% Pregnant
1 (vehicle control)	80
2 (10 mg/kg)	100
3 (50 mg/kg)	100
4 (100 mg/kg)	60

The mean number of corpora lutea and implantations and the mean implantation efficiency of the low- and mid-dose groups were comparable to or slightly higher than those of the control group (please refer to Table 1 below). In the one surviving pregnant high-dose rat, a comparable implantation efficiency was noted with the control group.

Higher, but not statistically significant, mean incidences of resorptions and lower mean incidences of fetal viability were noted in the low- and mid-dose groups when compared to the control group. (Please refer to Table 2 below.) Complete resorption was noted in the one surviving pregnant high-dose rat. One fetal death was noted in a mid-dose female.

Table 1

	Dose Group (mg/kg)			
	0	10	50	100
Mean Number of:				
Corpora lutea	14.3	15.6	13.0	19.0
Implantations	11.5	12.4	11.0	15.0
Resorptions	0.3	1.0	1.8	15.0
Fetuses - dead	0.0	0.0	0.2	0.0
- alive	11.3	11.4	9.0	0.0

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Table 2

Indices Calculated on a Per Litter Per Group Basis	Dose Group (mg/kg) 0	10	50	100
Mean Implantation Efficiency (%)	80.8	80.4	84.8	79.0
Mean Incidence of Resorption (%)	2.3	8.6	16.8	100.0
Mean Incidence of Fetal Death (%)	0.0	0.0	2.0	0.0
Mean Incidence of Fetal Viability (%)	97.8	91.4	81.2	0.0

Comparable mean fetal body weights and lengths were noted between the control and the low-dose groups. A lower mean weight and length was noted in the mid-dose group, with the mean weight being statistically significantly lower when compared to the control group.

Gross visceral examination revealed one edematous mid-dose fetus and one slightly edematous mid-dose fetus.

Discussion and Conclusion: The report indicated that the data did reveal a dose response, with the low-dose group exhibiting no maternal and minimal (if any) fetal toxicity. On the basis of the data and a dose response, dosage levels were chosen for a larger teratology study.

NOEL: None selected or suggested.

Maximum Tolerated Dose (MTD) for pregnant dams appears to be 50 mg/kg.

Classification: Supplementary